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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/917,330	07/27/2001	Satoshi Aotsuka	TOYAM75.001AUS	1241

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EXAMINER	
GALITSKY, NIKOLAI M	
ART UNIT	**PAPER NUMBER**
1631	4

DATE MAILED: 05/24/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/917,330	AOTSUKA, SATOSHI	
	Examiner	Art Unit	
	Nikolai M Galitsky	1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-3 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-3 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) [redacted] sheet 1 .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION.

The art unit designated for this application has changed. Applicant(s) are hereby informed that future correspondence should be directed to Art Unit 1631.

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a) (1) and (a) (2). This application fails to comply with the requirements of 37 C.F.R. § 1.821 through 1.825. Applicant has numerous sequences in the specification, which fall under the sequence rules, which do not have SEQ ID NOs therewith. See, for example, that Table 1, page 32 in specification has sequences, which are not in the sequence listing. All requirements regarding statements should be renewed along with the new sequence listing. Therefore, applicant is required to submit the following:

1. As a separate part of the disclosure on paper copy or compact disk copy, a "Sequence Listing" as 37 C.F.R. § 1.821(c).
2. A copy of the "Sequence Listing" in computer readable form as required by 37 C.F.R. § 1.821 (e).
3. A statement that the content of the paper and computer readable copies are the same and include no new matter, as required by 37 C.F.R. § 1.821 (f) and 37 C.F.R. § 1.821 (g).
4. All sequences in the specification, which fall under the sequence rules, must have a SEQ ID NO next to each citation of the sequence in the specification.

Applicant is given the same response time regarding this failure to comply as that set forth to respond to this office action. A complete response to this office action includes compliance with this sequence rule compliance requirement. Failure to comply may result in abandonment of this application.

Claim Rejections - 35 USC § 112, second.

The following is a quotation of the second paragraph of 35 U.S.C. 112:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-3 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Abbreviations such as "PCR", in instant claims 1 and 2 render claims vague and indefinite unless either replaced by or supplemented with the full names thereof. Clarification of the claims is requested via clearer claim wording.

In claim 1, line 26, an "oligomers should act as primers" which is hybridized to target sequence. The word "primers" suggests that it may be extended by a polymerase enzyme in step 2) of claim 1 by performing PCR. No limitation is directed to such extension practice in claim 1. Do applicants intend that the primer wording in the claim require the formation of an extension product via a polymerase enzyme activity? Clarification is requested via clearer claim wording as to the metes and bounds of the modification practice in step 2) as noted above.

In claim 2, line 29, these "oligomers should act as primers" which is hybridized to target sequence. The word "primers" suggests that it may be extended by a polymerase enzyme in step 2) of claim 1 by performing PCR. No limitation is directed to such extension practice in

claim 1. Do applicants intend that the primer wording in the claim require the formation of an extension product via a polymerase enzyme activity? Clarification is requested via clearer claim wording as to the metes and bounds of the modification practice in step 2) as noted above.

In claim 1, step 2), and elsewhere, the limitation "dividing a target sequence ... into 2N where N is a positive integer" suggests that N could go to the infinity. What will be the size of "sections" and designed "part or parts" that have a length that the nucleotide sequence of each part can specifically make base-pairing? Clarification is requested via clearer claim wording as to the metes and bounds of the dividing practice in step 1) as noted above.

In claim 2, step 2), and elsewhere, the limitation "dividing a target sequence ... into 2^n where n is a positive integer" suggests that n could go to the infinity. What will be the size of "sections" and designed "part or parts" that have a length that the nucleotide sequence of each part can specifically make base-pairing? Clarification is requested via clearer claim meaning as to the metes and bounds of the dividing practice in step 1) as noted above.

Claim Rejections - 35 USC § 103(a)

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Although the invention is not identically disclosed or described as set forth in 35 U.S.C. 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was

made to a designer having ordinary skill in the art to which said subject matter pertains, the invention is not patentable.

Claims 1-2 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stemmer et al. (Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides. Gene. 1995, Volume 164, Issue 1, pages 49-53), as applied to claims 1-2 above, and further in view of Sandhu et al. (Dual Asymmetric PCR: One-Step Construction of Synthetic Genes. BioTechniques. 1992, Volume 12, Number 1, pages 14-16).

The claimed invention relates to a method for producing DNA by preparing overlapping oligomers, performing PCR by using the oligomers, sequencing synthesized DNAs and repeating PCR by using the selected DNA. That is imposed on the methods based on PCR.

Stemmer et al. describes assembly PCR as a method for the synthesis of long DNA sequences from large numbers of oligodeoxyribonucleotides (oligos). The method, which is derived from DNA shuffling, does not rely on DNA ligase but instead relies on DNA polymerase to build increasingly longer DNA fragments during the assembly process. A 1.1-kb fragment containing the TEM-1 b-lactamase-encoding gene (bla) was assembled in a single reaction from a total of 56 oligos, each 40 nucleotides (nt) in length [claims 1, step(1) and 2, step (1)]. The synthetic gene was PCR amplified [claims 1, step(2) and 2, step (2)] and cloned in a vector containing the tetracycline-resistance gene (TcR) as the sole selectable marker. Without relying on ampicillin (Ap) selection, 76% of the TcR colonies were ApR, making this approach a general method for the rapid and cost-effective synthesis of any gene. We tested the range of assembly PCR by synthesizing, in a single reaction vessel containing 134 oligos, a high-molecular-mass multimeric form of a 2.7-kb plasmid containing the bla gene, the a-

fragment of the lacZ gene and the pUC origin of replication. Digestion with a unique restriction enzyme, followed by ligation and transformation in Escherichia coli, yielded the correct plasmid. Assembly PCR is well suited for several in vitro mutagenesis strategies (Abstract).

Claim 1, step (1) is directed to the dividing a target sequence, which is a nucleotide sequence of DNA to be synthesized; step (2) is directed to performing PCR; step (3) is directed to the sequencing synthesized DNAs and step (4) is directed to the repeating steps.

Stemmer et al. describes the assembly PCR protocol consists of four steps. Three of these steps are those of instant invention:

- oligo synthesis [Claim 1, step (1) of the instant invention] and see Figure 1(a) on the page 50, column 2, by Stemmer et al.,
- gene assembly [which is similar to the claim 1, step (2)of the instant invention],
- gene amplification [claim 1, step (4) of the instant invention].

Also, Stemmer et al. describes all plasmids contained the five restriction sites characteristic for the synthetic gene construct. The *bla* gene from one of these plasmids was sequenced [claim 1, step (3) and claim 2, step (4)] : three point mutations were found presumably introduced during the PCR amplification process (Page 50, column 1, lines34-37). The experiments described in this paper were designed to demonstrate that the gene assembly method can be used for the construction of any gene as long as the gene product it codes for be identified. (Page 52, column 1, lines 4-7).

Stemmer et al noted that the real advantage of DNA shuffling as described in this paper lies in the robustness of the method: (i) all oligos used for gene synthesis were 40-mers, which can be synthesized efficiently without the need for further purification: (ii) we did not give special

consideration to differences in melting temperature of the overlapping regions, which was fixed at 20 bp in length ; (iii) the number of oligos that can be used in the one-step DNA assembly process is (surprisingly) high (Page 52, column 2, lines2-11). This method will allow for the concomitant synthesis of many different oligos. Combined with assembly PCR, it will become possible to build very complex DNAs from off-the-shelf chemicals in a cost-effective way (Page 52, column 2, lines 22-26).

Stemmer et al. teaches assembly PCR as a method for the synthesis of long DNA sequences from large numbers of oligodeoxyribonucleotides (oligos), however, Stemmer et al. fails to describe ratio of the reaction mixture.

Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sandhu et al.

Sandhu et al. described a one-step process for constructing synthetic genes. Four adjacent oligonucleotides 17-100 bases in length short overlaps of 15-17 bases used as primers in a PCR mixture (Abstract, page 14, column 2, lines 1-6). Sandhu et al. reasoned that if four overlapping oligonucleotides were mixed and annealed in the presence of buffer, nucleotides and *Taq* polymerase, than the reactions shown in (Page 15, Figure 1) would occur under PCR conditions. By using an asymmetric ratio of nucleotides in which the two internal oligonucleotides, H2 and H3, are limiting, we bias the system to make double- and single-strand intermediates of the type shown in Figure 1 step b. These intermediates anneal and prime the synthesis of full-length, double-strand molecules (step) that can then be amplified (step d) because of excess H1 and H4 oligonucleotid primers. To see if the intermediates predicted in Figure 1 occurred with kinetics postulated, we set up a series of identical reactions and analyzed them after a total of 5, 10, 15, 20, and 25 cycles of amplification (page15, column 1, lines1-22).

The reaction run in (page 15, column 3, Figure 2) was repeated.... Two isolates were analyzed by DNA sequencing and found to have the sequence of the component oligonucleotides (Page 15, column 3, lines 2-7).

Further, Sandhu et al. teaches synthetic oligonucleotides 100 bases long can be routinely synthesized in reasonable yields. Using this method with 100-mers having 15-bp overlaps, a 355-bp duplex could be generated. If two 355-mers overlapping by 15 bp were made in separate reactions and then mixed, in principle they could be amplified as a continuous 695-mer using the appropriate rightmost and leftmost priming sequences. Thus, one could grow a synthetic gene of quite large proportions ultimately being limited by effective distance *Taq* polymerase can amplify under PCR conditions (page 15, column 3, lines 16-23 and page 16, column 1, lines 1-8).

Sandhu et al. noted the overall advantages of this method are a reduction in steps and time needed to make duplex oligonucleotides... Clearly, it also represents a straightforward way for production of gene with any mutation desired (page 16, column 1, lines 8-16).

Thus, it would have been obvious to some one of ordinary skill in the art at the time of the instant invention to use an asymmetric ratio of nucleotides motivated by Sandhu et al. to additionally practice an assembly PCR as a method for the synthesis of long DNA sequences from large numbers of oligodeoxyribonucleotides, thus resulting in the practice of the instant invention.

No claim is allowed.

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the PTO Fax Center located

in Crystal Mall 1. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CFR § 1.6(d)). The CM1 Fax Center number is either (703) 308-4242 or (703) 305-3014.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Nikolai M Galitsky, Ph.D., whose telephone number is (703) 308-2422. The examiner can normally be reached on Monday-Friday from 9 A.M. to 5 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Woodward, Ph.D., can be reached on (703) 308-4028.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Bill Phillips, whose telephone number is (703) 305-3482 or to the Technical Center receptionist whose telephone number is (703) 308-0196.

May 15, 2002

NG

ARDIN H. MARSCHEL
PRIMARY EXAMINER